

13



Untitled
SEQUENCE LISTING

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<120> Modifications of Apoptin

<130> 2906-4996.1

<140> 10/083,849

<141> 2001-10-19

<150> US 60/242,397

<151> 2000-10-20

<160> 20

<170> PatentIn version 3.1

<210> 1

<211> 121

<212> PRT

Untitled

<213> Chicken anemia virus

<220>

<221> MISC_FEATURE

<222> (1)..(121)

<223> Apoptin (a small protein derived from chicken anemia virus) encoded by pCMV-Vp3 and by GFP-Apoptin constructs

<400> 1

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

Untitled

<210> 2

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> Apoptin protein encoded by pIRESneo alanine mutants

<220>

<221> MISC_FEATURE

<223> Differs from Apoptin protein encoded by pCMV-Vp3 and by GFP-Apo
pt
in constructs by replacement of the arginine residue at position
n
116 with a lysine residue

<400> 2

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Untitled

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Lys Arg Arg Ile Arg Leu
115 120

<210> 3

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)...(121)

<223> mutant Ala(5)-86 of 5-alanine linker-scanning mutant series of
Ap
optin

<400> 3

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Untitled

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Ala Ala Ala Ala Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 4

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-91 of 5-alanine linker-scanning mutant series of
Ap
optin

Untitled

<400> 4

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Ala Ala Ala Ala Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 5

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

Untitled

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-96 of 5-alanine linker-scanning mutant series of
Ap
optin

<400> 5

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Ala
85 90 95

Ala Ala Ala Ala Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 6

<211> 121

Untitled

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-101 of 5-alanine linker-scanning mutant series of
A
poptin

<400> 6

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Ala Ala Ala Ala Thr Thr Thr Pro Ser Arg Pro
100 105 110

Untitled

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 7

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-106 of 5-alanine linker-scanning mutant series of
A
poptin

<400> 7

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Untitled

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Ala Ala Ala Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 8

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-111 of 5-alanine linker-scanning mutant series of
A
poptin

<400> 8

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Untitled

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Pro Ser Ala Ala
100 105 110

Ala Ala Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 9

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> mutant Ala(5)-116 of 5-alanine linker-scanning mutant series of
A
poptin

<400> 9

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Untitled

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val	Ser	Glu	Leu	Lys	Glu	Ser	Leu	Ile	Thr	Thr	Thr	Pro	Ser	Arg	Pro
100								105							110

Arg Thr Ala Ala Ala Ala Ala Ala Leu
115 120

<210> 10

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1) .. (121)

<223> single point mutant T106A of Apoptin

Untitled

<400> 10

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 11

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

Untitled

<222> (1)..(121)

<223> single point mutant T107A of Apoptin

<400> 11

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 12

<211> 121

<212> PRT

<213> Chicken anemia virus

Untitled

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108A of Apoptin

<400> 12

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Ala Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 13

Untitled

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant P109A of Apoptin

<400> 13

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Thr Ala Ser Arg Pro
100 105 110

Untitled

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 14

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T106E of Apoptin

<400> 14

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Untitled

Val Ser Glu Leu Lys Glu Ser Leu Ile Glu Thr Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 15

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T107E of Apoptin

<400> 15

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Untitled

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Glu Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 16

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> single point mutant T108E of Apoptin

<400> 16

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Untitled

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Thr Glu Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 17

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutation T106A107A of Apoptin

<400> 17

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Untitled

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Ala Thr Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 18

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

<222> (1)..(121)

<223> double point mutant T107A108A of Apoptin

Untitled

<400> 18

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Thr Ala Ala Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 19

<211> 121

<212> PRT

<213> Chicken anemia virus

<220>

<221> MUTAGEN

Untitled

<222> (1)..(121)

<223> double point mutant T106A108A of Apoptin

<400> 19

Met Asn Ala Leu Gln Glu Asp Thr Pro Pro Gly Pro Ser Thr Val Phe
1 5 10 15

Arg Pro Pro Thr Ser Ser Arg Pro Leu Glu Thr Pro His Cys Arg Glu
20 25 30

Ile Arg Ile Gly Ile Ala Gly Ile Thr Ile Thr Leu Ser Leu Cys Gly
35 40 45

Cys Ala Asn Ala Arg Ala Pro Thr Leu Arg Ser Ala Thr Ala Asp Asn
50 55 60

Ser Glu Ser Thr Gly Phe Lys Asn Val Pro Asp Leu Arg Thr Asp Gln
65 70 75 80

Pro Lys Pro Pro Ser Lys Lys Arg Ser Cys Asp Pro Ser Glu Tyr Arg
85 90 95

Val Ser Glu Leu Lys Glu Ser Leu Ile Ala Thr Ala Pro Ser Arg Pro
100 105 110

Arg Thr Ala Arg Arg Arg Ile Arg Leu
115 120

<210> 20

<211> 8

<212> PRT

<213> Chicken anemia virus

Untitled

<220>

<221> MISC_FEATURE

<223> amino acid sequence encoding the SV40-Large T nuclear localizat
io
n signal

<400> 20

Pro Pro Lys Lys Lys Arg Lys Val
1 5